AR201-13822B

03-June-2002

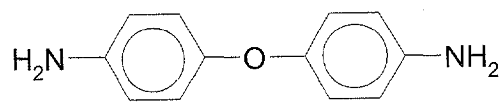
The studies listed below were selected to represent the best available study design and execution for these HPV toxicity endpoints. Other data of equal or lesser quality are not summarized, but are listed as related references in this document.

1.0 Substance Information

CAS Number: 101-80-4

Chemical Name: Benzeneamine, 4,4'-oxybis

Structural Formula:



Other Names:

4,4'-Oxydianiline
p,p'-Oxydianiline
Oxydianiline
ODA
4-Aminophenyl ether
Bis(p-aminophenyl) ether
4,4'-Diaminodiphenyl ether
Oxybis(4-aminobenzene)
p,p'-Oxybis(aniline)
p-Aminophenyl ether
Bis(4-aminophenyl) ether
Dadpe
4,4-Dadpe
4,4'-Diaminodiphenyl oxide
Diaminodiphenyl ether
p,p'-Diaminodiphenyl ether
4,4'-Diaminobiphenyl ether
4,4'-Oxybis(aniline)
4,4-Oxydianiline
4,4'-Oxydiphenylamine
Oxydi-p-phenylenediamine
4,4'-Diaminophenyl ether
4,4'-Diaminophenyl oxide

Exposure Limits:

0.1 mg/m^3, 8-hour TWA: DuPont Acceptable Exposure Limit (AEL)

0.3 mg/m^3, 15-minute TWA: DuPont AEL

5 mg/m^3, 8-hour TWA (respirable dust): OSHA Permissible Exposure Limit (PEL)

15 mg/m^3, 8-hour TWA (total dust): OSHA PEL

5 mg/m^3: Russia Occupational Exposure Limit (OEL)

2.0 Physical/Chemical Properties

2.1 Melting Point

Value:	186-187ºC
Decomposition:	No Data
Sublimation:	No Data
Pressure:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Dean, J. A. (1985). Lange's Handbook of Chemistry, 13th ed., McGraw Hill Book Co., New York, NY (NISC/EF-0007595).
Reliability:	Not assignable because limited study information was available.

Additional Reference for Melting Point:

DuPont Co. (1994). Material Safety Data Sheet No. DU000275 (August 31).

2.2 Boiling Point

Value:	350ºC
Decomposition:	No Data
Pressure:	No Data
Method:	Estimated by PCCHEM-PCGEMS
GLP:	Not Applicable
References:	SRC (Syracuse Research Corporation) (1988). Syracuse Research Corporation Calculated Values (NISC/EF-0007596).
Reliability:	Estimated value based on accepted model.

Additional Reference for Boiling Point:

DuPont Co. (1994). Material Safety Data Sheet No. DU000275 (August 31).

Kazinik, E. M. et al. (1971). Zh. Anal. Khim., 26:154-157 (cited in WHO (World Health Organization (1982). IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 29, pp. 203-212, International Agency for Research on Cancer, France).

2.3 Density

Value:	0.46-0.54 (Bulk density; loose)
Temperature:	No Data
Method:	No Data
GLP:	Unknown
Results:	No additional data.
Reference:	DuPont Co. (1994). Material Safety Data Sheet No. DU000275 (August 31).
Reliability:	Not assignable because limited study information was available.

Additional References for Density: None Found.

2.4 Vapor Pressure

Value:	3.07×10^{-7} mm Hg
Temperature:	25°C
Decomposition:	No Data
Method:	Estimated by PCCHEM-PCGEMS
GLP:	Not Applicable
Reference:	GEMS (1987). Graphical Exposure Modeling System, PCCHEM, U. S. EPA (HSDB/1316).
Reliability:	Estimated value based on accepted model.

Additional Reference for Vapor Pressure:

DuPont Co. (1994). Material Safety Data Sheet No. DU000275 (August 31).

2.5 Partition Coefficient (log Kow)

Value:	2.06
Temperature:	No Data
Method:	Estimated by CLOG-PCGEMS
GLP:	Not Applicable
Reference:	SRC (Syracuse Research Corporation) (1988). Syracuse Research Corporation Calculated Values (NISC/EF-0007590)
Reliability:	Estimated value based on accepted model.

Additional References for Partition Coefficient (log Kow):

DuPont Co. (1994). Material Safety Data Sheet No. DU000275 (August 31).

Syracuse Reseach Corporation KOWWIN Program v1.66.

2.6 Water Solubility

Value:	139 ppm
Temperature:	25°C
pH/pKa:	No Data
Method:	Calculated
GLP:	Not Applicable
Reference:	SRC (Syracuse Research Corporation) (1988). Syracuse Research Corporation Calculated Values (NISC/EF-0007589).
Reliability:	Estimated value based on accepted model.

Additional Reference for Water Solubility:

DuPont Co. (1994). Material Safety Data Sheet No. DU000275 (August 31).

2.7 Flash Point

Value:	219ºC
Method:	SFCC
GLP:	Unknown
Reference:	DuPont Co. (1994). Material Safety Data Sheet No. DU000275 (August 31).
Reliability:	Not assignable because limited study information was available.

Additional References for Flash Point: None Found.

2.8 Flammability

Results:	Autoignition Temperature = 490ºC
Method:	No Data
GLP:	Unknown
Reference:	DuPont Co. (1994). Material Safety Data Sheet No. DU000275 (August 31).
Reliability:	Not assignable because limited study information was available.

Additional References for Flammability: None Found.

3.0 Environmental Fate

3.1 Photodegradation

Concentration:	Not Applicable
Temperature:	Not Applicable
Direct Photolysis:	Not Applicable
Indirect Photolysis:	Not Applicable
Breakdown Products:	Not Applicable
Method:	Based upon an estimated vapor pressure of 3.07×10^{-7} mm Hg at 25°C (GEMS, 1987), 4,4'-oxydianiline is expected to exist in both the vapor and particulate phases in the ambient atmosphere (Eisenreich et al., 1981). Vapor phase 4,4'-oxydianiline is degraded rapidly in an average ambient atmosphere by reaction with photochemically produced hydroxyl radicals at an estimated half-life of about 1.8 hours (Atkinson, 1987). Particulate phase 4,4'-oxydianiline may be removed from the atmosphere via dry deposition (SRC, n.d.).
GLP:	Not Applicable
References:	GEMS (1987). Graphical Exposure Modeling System, PCCHEM, U. S. EPA (HSDB/1316). Eisenreich, S. J. et al. (1981). <u>Environ. Sci. Technol.</u>, 15:30-38 (HSDB/1316). Atkinson, R. (1987). <u>J. Inter. Chem. Kinet.</u>, 19:799-828 (HSDB/1316). SRC (Syracuse Research Corporation) (n.d.). (HSDB/1316).
Reliability:	Estimated value based on accepted model.

Additional Reference for Photodegradation:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

SRC (Syracuse Research Corporation) (n.d.). AOP Computer Program v1.90 Estimated Values.

3.2 Stability in Water

Concentration:	Not Applicable
Half-life:	The Henry's Law constant for 4,4'-oxydianiline is estimated to be $5.82x10^{-10}$ atm-m^3/mole (Henry v3.10 Program, Bond SAR Method in SRC Epiwin v3.05). This Henry's Law constant indicates that 4,4'-oxydianiline will not volatilize rapidly from water surfaces. The estimated volatilization half-life from a model river (1 m deep, flowing 1 m/sec, wind velocity of 5 m/sec) is approximately $7.41x10^5$ days (Epiwin v3.05). The estimated volatilization half-life from a model lake (1 m deep, flowing 0.05 m/sec, wind velocity of 0.5 m/sec) is approximately $8.1x10^6$ days (Epiwin v3.05). By analog to other aromatic amines (Parris, 1980), 4,4'-oxydianiline may undergo covalent bonding with humic materials in the water column and in sediment. Partitioning from the water column to sediment and suspended material may therefore be an important removal process from water. 4,4'-Oxydianiline in the water column may be susceptible to photooxidation via hydroxyl and peroxy radicals based on analogy to other aromatic amines (Mill and Mabey, 1985). Furthermore, aquatic hydrolysis does not appear to be an environmentally important removal process for 4,4'-oxydianiline in water (SRC, n.d.).
% Hydrolyzed:	Not Applicable
Method:	Modeled Data: Syracuse Research Corporation Epiwin v3.05.
GLP:	Not Applicable
References:	SRC (n.d.). Syracuse Research Corporation (HSDB/1316). Parris, G. E. (1980). Environ. Sci. Technol., 14:1099-1106 (HSDB/1316). Mill, T. and W. Mabey (1985). Environmental Exposure from Chemicals, Neely, W. R. and G. E. Blau (eds.), Vol. 1, pp. 208-211, CRC Press, Boca Raton, FL (HSDB/1316).
Reliability:	Estimated value based on accepted model.

Additional References for Stability in Water: None Found.

3.3 Transport (Fugacity)

Media:	Air, Water, Soil, and Sediments	
Distributions:	Air:	0.0247%
	Water:	31.2%
	Soil:	68.6%
	Sediment:	0.12%
Half-life:	Air:	1.284 hours
	Water:	900 hours
	Soil:	900 hours
	Sediment:	3600 hours
Adsorption Coefficient:	Not Applicable	
Desorption:	Not Applicable	
Volatility:	Not Applicable	

Method: Calculated according to Mackay, Level III, Syracuse Research Center Epiwin Version 3.05. Emissions (1000 kg/hr) to air, water, and soil compartments using EPA model defaults.

Data Used:
Molecular Weight: 200.24
Henry's Law Constant: 5.82×10^{-10} atm-m^3/mole (Henrywin Program, Group SAR method)
Vapor Pressure: 3.07×10^{-7} mm Hg (Mpbpwin program)
Log Kow : 2.06 (Kowwin program)
Soil Koc : 47.1 (calculated by model)

GLP: Not Applicable

References: Syracuse Research Corporation Epiwin v3.05 contains a Level III fugacity model. The methodology and programming approach was developed by Dr. Donald Mackay and co-workers and are detailed in:

Mackay, D. (1991). Multimedia Environmental Models; The Fugacity Approach, pp. 67-183, Lewis Publishers, CRC Press.

Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1618-1626.

Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1627-1637.

Reliability: Estimated value based on accepted model.

Additional Reference for Transport (Fugacity):

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

SRC (Syracuse Research Corporation) (1988). Syracuse Research Corporation Calculated Values (NISC/EF-0007592).

3.4 Biodegradation

Value: Laboratory studies indicate that 4,4'-oxydianiline is partially biodegradable, and it is noninhibitory to biological populations. Further results are listed below:

BOD = 0.52
COD (theoretical) = 2.08
COD (measured) = 1.62
BOD/COD = 0.32
Incremental O_2 uptake in 14 days = 27 ppm.

Breakdown Products: No Data

Method: Phase 1: Acclimation Tests and COD/BOD Determinations: A laboratory scale biological treatment unit was operated to separate acclimated seed microorganisms to the test substance. Both effluent and settled suspended solids from a wastewater treatment lagoon were used as the seed population. The unit received nutrient enhanced wastewater (influent to the lagoon) for 1 week. After 1 week, the influents were supplemented with the test substance. Supplemental concentrations were increased incrementally from 1 to 10 ppm throughout the 3-week acclimation period. Analyses for chemical oxygen demand (COD) and biochemical oxygen demand (BOD) were performed regularly on the influent and effluent. Measurements for mixed liquor (biomass) were also collected.

Phase 2: Respirometer Tests: An electrolytic respirometer test (E/BOD) was performed to determine the potential biodegradability and inhibitory characteristics of the test substance. The E/BOD test was conducted in a closed cell using acclimated microbial populations from Phase 1 reactor. Duplicate tests were conducted. One feed control unit was included, which contained acclimated seed microorganisms, wastewater, nitrogen, and a pH buffer. Tests were run for at least 14 days to allow sufficient time

	for potential biochemical oxidation to be complete.
GLP:	Unknown
Reference:	DuPont Co. (1990). Unpublished Data (February).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Biodegradation: None Found.

3.5 Bioconcentration

Value:	BCF = approximately 22 (log BCF = 1.34). This BCF value suggests 4,4'-oxydianiline will not bioconcentrate in aquatic organisms (SRC, n.d.)
Method:	The BCF was estimated based on an estimated log Kow of 2.06 (GEMS, 1987) and a regression derived equation (Lyman et al., 1990).
GLP:	Not Applicable
References:	Lyman, W. J. et al. (1990). Handbook of Chemical Property Estimation Methods, pp. 4-9, 5-4, 5-10, 7-4, 7-5, and 15-15 to 15-32, American Chemical Society, Washington, DC (HSDB/1316). GEMS (1987). Graphical Exposure Modeling System, PCGEMS (HSDB/1316). SRC (Syracuse Research Corporation) (n.d.). (HSDB/1316).
Reliability:	Estimated value based on accepted model.

Additional References for Bioconcentration: None Found.

4.0 Ecotoxicity

4.1 Acute Toxicity to Fish

Type:	**24-hour Toxicity**
Species:	*Ptychocheilus oregonensis* (Northern squawfish) *Oncorhynchus tshawytscha* (Chinook salmon) *Oncorhynchus kisutch* (Coho salmon, silver salmon)
Value:	> 10 ppm
Method:	The fish used measured 5-10 cm. A series of insulated, round, stainless steel tubs were used for water baths. The water was obtained from Rochat Creek, and a chemical analysis of the water was made during summer when the stream flows were low. The pH of the water was 7.2, alkalinity was 7 ppm, and hardness was 0-17 ppm. The baths were served by a common refrigerated reservoir through which temperature-controlled water was

	recirculated. Each tub held 9.5 L plastic aquaria, and each aquarium was aerated by a single stone air-breaker and lined with a disposable polyethylene poultry bag. The bag was closed at the top to prevent fish from escaping. Fish were acclimated at about the temperatures of the assay vessels. The acclimation varied from 3-24 hours, but most fish were conditioned at least overnight. The test fish were starved during acclimatization and transferred to the assay vessel approximately 2 hours prior to addition of 10 ppm of test substance. Usually 1 squawfish and 1 individual of each of 2 species of salmonid were placed together in 1 vessel in 4 L of water, the loading being approximately 5 g of fish/L solution. Water temperature was taken several times during each test, with only the highest temperature reported in a 24-hour test period. The time at which a fish lost its equilibrium and time of death were recorded. Loss of equilibrium was defined as when a fish was no longer able to remain right-side-up, and death was designated when a fish ceased visible movement.
GLP:	No
Test Substance:	4,4’-Oxydianiline, purity not specified
Results:	Neither death nor loss of equilibrium occurred in *Ptychocheilus oregonensis*, *Oncorhynchus tshawytscha*, or *Oncorhynchus kisutch* at 10 ppm.
Reference:	MacPhee, C. and R. Ruelle (1969). Univ. of Idaho Forest, Wildl. Range Exp. Station Bull. No. 3, Moscow, ID.
Reliability:	Low because an inappropriate method or study design was used.

Type:	**96-hour LC_{50}**
Species:	Fish
Value:	330 mg/L ($\log_{10}$ Kow of 1.0) 54.4 mg/L ($\log_{10}$ Kow of 2.06) 11 mg/L ($\log_{10}$ Kow of 3.0)
Method:	Modeled
GLP:	Not Applicable
Test Substance:	4,4’-Oxydianiline
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User’s Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional Reference for Acute Toxicity to Fish:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Applegate, V. C. et al. (1957). Toxicity of 4346 Chemicals to Larval Lampreys and Fishes, United States Department of the Interior, Washington, DC.

4.2 Acute Toxicity to Invertebrates:

Type:	**48-hour LC_{50}**
Species:	*Daphnia magna*
Value:	0.92 mg/L (0.84-1.01 mg/L)
Method:	Procedures used in the acute toxicity test closely followed those described in the MIC Environmental Assessment Method for Conducting Acute Toxicity Tests with *Daphnia magna* (Grueber and Adams, 1980), and Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians (U.S. EPA, 1975). The static toxicity tests were conducted in 250 mL beakers that contained 200 mL of test solution. The dilution water used was a mixture of distilled deionized water and well water from St. Peters, MO. The well water was diluted with distilled water to provide a hardness of approximately 60 ppm. For each test concentration, the test substance, dissolved in dimethylformamide, was injected into dilution water using a microliter syringe and stirred vigorously for 3-5 minutes. The solution was then divided into aliquots in triplicate beakers to provide appropriate replication. The remaining solution was used for 0-hour dissolved oxygen, pH, alkalinity, and hardness determinations. A control, consisting of the same dilution water and conditions, but with no test substance was used, as was a solvent control. Nominal test concentrations were 0 (control), 0 (solvent control), 0.15, 0.31, 0.62, 1.25, 2.5, and 5 mg/L. All test vessels were maintained at room temperature. Test solutions were not aerated during the test. Ten daphnids were randomly assigned to each test vessel within 30 minutes after the test substance was added, for a total of 30 daphnids per concentration. Dissolved oxygen, pH, alkalinity, hardness, and temperature of the controls and high concentration were monitored at the test initiation. At

	the test conclusion, these parameters were measured in the controls and low-, medium-, and high-test concentrations.
GLP:	Unknown
Test Substance:	4,4'-Oxydianiline, purity >99%
Results:	During the 48-hour toxicity tests, the pH and dissolved oxygen ranged from 7.8-8.4 and 7.9-9.0 mg/L, respectively. Temperatures ranged from 21.9-23.8°C. Alkalinity and hardness ranged from 78-100 and 60-72 mg/L, respectively. Mortality ratios were 2/30, 1/30, 0/30, 2/30, 1/30, 27/30, 30/30, and 30/30 at 0 (control), 0 (solvent control), 0.15, 0.31, 0.62, 1.25, 2.5, and 5 mg/L, respectively. The no observed effect concentration (NOEC) at 48 hours was 0.62 mg/L. The 24-hour LC_{50} was 4.52 mg/L (3.60-6.60 mg/L).
Reference:	Monsanto Co. (1986). Report No. MSL-5970, ESC-EAG-86-84 (cited in TSCA fiche OTS0546071). Grueber, D. J. and W. J. Adams (1980). Environmental Sciences Report ES-80-M-6. U.S. EPA (1975). Ecological Research Series, EPA 600/3-75-009, 61 pp.
Reliability:	Medium because a suboptimal study design was used. Only nominal test concentrations were used.

Type:	**48-hour EC_{50}**
Species:	Daphnid
Value:	2.6 mg/L (log_{10} Kow of 1.0) 1.3 mg/L (log_{10} Kow of 2.06) 0.7 mg/L (log_{10} Kow of 3.0)
Method:	Modeled
GLP:	Not Applicable
Test Substance:	4,4'-Oxydianiline
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC; prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Invertebrates: None Found.

4.3 Acute Toxicity to Aquatic Plants

Type:	**96-hour ChV**
Species:	Algae
Value:	20 mg/L (log_{10} Kow of 1.0) 4.8 mg/L (log_{10} Kow of 2.06) 1.3 mg/L (log_{10} Kow of 3.0)
Method:	Modeled
GLP:	Not Applicable
Test Substance:	4,4'-Oxydianiline
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC; prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Aquatic Plants: None Found.

5.0 Mammalian Toxicity

5.1 Acute Toxicity

Type:	**Oral ALD**
Species/Strain:	Rats/Crl-CD
Value:	1500 mg/kg
Method:	4,4'-Oxydianiline was administered as a suspension in peanut oil in single doses to male rats (1/dose level) via intragastric intubation at doses of 40, 60, 90, 200, 300, 450, 670, 1000, 1500, 2250, 3400, or 5000 mg/kg. Survivors were killed 9-13 days later and were examined for pathologic changes. In addition, 3 animals were dosed at levels of 1500, 3400, and 5000 mg/kg and killed, when moribund, for pathologic evaluation.
GLP:	No
Test Substance:	4,4'-Oxydianiline, purity approximately 100%
Results:	Mortality was 0/1, 0/1,0/1, 0/1, 0/1, 0/1, 0/1, 0/1, 1/1, 1/1, 1/1, 1/1 at 40, 60, 90, 200, 300, 450, 670, 1000, 1500, 2250, 3400, and 5000 mg/kg, respectively. All deaths occurred within 12 days. Clinical signs of toxicity included discomfort (≥200 mg/kg), inactivity (≥670 mg/kg), glassy and pale eyes (≥670 mg/kg), prostration (≥2250 mg/kg), slow shallow respiration (≥2250 mg/kg), salivation (3400 mg/kg), lacrimation (≥2250 mg/kg), tremors (1500 mg/kg),

	convulsive movements of the head (5000 mg/kg), incoordination (1500 mg/kg), hair loss (200, 300, 450, 670, 1000, and 1500 mg/kg), bulging eyes (3400 and 5000 mg/kg), and ruffled fur (670 and 1000 mg/kg). In addition, weight loss was observed at 60, 200, 300, 450, 670, 1000, 1500, 3400, and 5000 mg/kg. Pathological changes at lethal doses included congestion of viscera in the one animal (2500 mg/kg); all others could not be observed due to advanced post-mortem changes. In the animals that were dosed, and killed when moribund, pathological changes included slightly brown blood and/or tissues (1500 and 5000 mg/kg), stomach distended with food ($\geq$3400 mg/kg), liver injury (1500 mg/kg), and spleen and adrenal gland congestion (1500 mg/kg). Pathological changes at non-lethal doses included liver injury (200 and 1000 mg/kg), kidney injury (1000 mg/kg), and extramedullary blood formation ($\geq$200 mg/kg). A single dose of 90 mg/kg caused no detectable injury.
Reference:	DuPont Co. (1962). Unpublished Data, Haskell Laboratory Report No. 9-62.
Reliability:	High because a scientifically defensible or guideline method was used.

Type:	**Oral LD_{50}**
Species/Strain:	Rat/Strain not specified
Value:	725 ± 50 mg/kg
Method:	4,4'-Oxydianiline was administered to rats (number and age not specified) intragastrically as a suspension in a 1% solution of starch mucilage. Observations were conducted for 15 days. The LD_{50} was calculated according to Kerber.
GLP:	No
Test Substance:	4,4'-Oxydianiline, purity not specified
Results:	The peroral administration of toxic doses had a constipating effect.
Reference:	Lapik, A. S. et al. (1968). Hyg. Sanit., 33(10):137-138.
Reliability:	Not assignable because limited study information was available.

Type:	**Oral LD_{50}**
Species/Strain:	Mice/Strain not specified
Value:	685 ± 50 mg/kg
Method:	4,4'-Oxydianiline was administered to mice (number and age not specified) intragastrically as a suspension in a 1% solution of starch mucilage. Observations were conducted for 15 days. The LD_{50} was calculated according to Kerber.
GLP:	No

Test Substance:	4,4'-Oxydianiline, purity not specified
Results:	No additional data.
Reference:	Lapik, A. S. et al. (1968). Hyg. Sanit., 33(10):137-138.
Reliability:	Not assignable because limited study information was available.

Additional References for Acute Oral Toxicity:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Lapik, A. S. et al. (1968). Hyg. Sanit., 33(10):137-138.

Lapik, A. S. and M. Dolgykh (1984). Izv. Sib. Otd. Akad. Nauk SSSr Ser. Biol. Nauk, (3):124-126 (CA102:199083g).

Makarenko, A. A. and S. A. Lapik (1968). Gigiena, 68:25-28.

NCI (National Cancer Institute) (1980). Technical Report Series No. 205, National Institutes of Health, Bethesda, MD.

Izmerov, N. F. (1982). Toxicometric Parameters of Industrial Toxic Chemicals Under Single Exposure, p. 43.

Data from these additional sources were not summarized because the study design was not adequate.

Griswold, D. P., Jr. et al. (1966). Cancer Res., 26(1):619-625.

Schafer, E. W., Jr. and W. A. Bowles, Jr. (1985). Arch. Environ. Contam. Toxicol., 14:111-129.

Type:	**Inhalation:** No Data.

Type:	**Dermal ALD**
Species/Strain:	Rabbit/Albino
Value:	> 5000 mg/kg
Exposure Time:	24 hours
Method:	The test substance was applied to the shaved skin of 1 rabbit as a 50% ointment in Carbowax 1500. The maximum feasible dose was 5000 mg/kg, but absorption of the test substance was poor. The animal was wrapped in moisture-proof cellophane and bandage for 24 hours. The rabbit was euthanized 12 days after the dermal application of the test substance.

GLP:	No
Test Substance:	4,4'-Oxydianiline, purity approximately 100%
Results:	No mortality was observed. Loss of appetite and weight loss for 5 days were observed. No pathological changes were observed.
Reference:	DuPont Co. (1962). Unpublished Data, Haskell Laboratory Report No. 9-62.
Reliability:	Medium because a suboptimal study design was used.

Additional Reference for Acute Dermal Toxicity:

Data from this additional source were not summarized because the vehicle used may have produced the adverse findings observed in the study.

DuPont Co. (1964). Unpublished Data, Haskell Laboratory Report 91-64.

Type:	**Dermal Irritation**
Species/Strain:	Guinea pig/Albino
Method:	4,4'-Oxydianiline, as a 10% solution in DMAC, was applied to the shaved intact skin of 10 albino guinea pigs. Observations were made after 24 hours of contact. A control test with dimethylacetamide (DMAC) was done on each animal in the same way.
GLP:	No
Test Substance:	4,4'-Oxydianiline (10% solution), purity not specified
Results:	The degree of irritation was the same as was seen in animals treated with DMAC alone. After 24 hours of contact, 4,4'-oxydianiline produced strong erythema in 3, mild eythema in 1, and no erythema in 6 animals. After 24 hours of contact, DMAC produced strong erythema in 3, moderate erythema in 3, mild erythema in 2, and no erythema in 1 animal.
Reference:	DuPont Co. (1964). Unpublished Data, Haskell Laboratory Report No. 91-64.
Reliability:	High because a scientifically defensible or guideline method was used.

Type:	**Dermal Irritation/Corrosion**
Species/Strain:	Rabbits/Albino
Method:	Six albino rabbits were clipped free of hair on the trunk and lateral areas and placed in FDA-type stocks. 4,4'-Oxydianiline (0.5 g) was placed on the trunk of the rabbits under a gauze pad. The trunk of each rabbit was then loosely wrapped with rubber sheeting. After 4 hours, the rabbits were removed from the stocks, washed, and reactions were read according to the system of the Federal Hazardous

	Substances Act. Readings were also made at 24 and 48 hours.
GLP:	No
Test Substance:	4,4'-Oxydianiline, purity 99.88%
Results:	No erythema or edema was observed at 4, 24, or 48 hours. Skin corrosion was not observed in any of the animals. According to the regulations of the Department of Transportation, 4,4'-oxydianiline was not considered a corrosive material.
Reference:	DuPont Co. (1973). Unpublished Data. DuPont Co. (1973). Unpublished Data, Haskell Laboratory Report No. 634-73.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Dermal Irritation:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Lapik, A. S. and M. P. Dolgykh (1984). Izv. Sib. Otd. Akad. Nauk SSSR Ser. Biol. Nauk, (3):124-126 (CA102:199083g).

Makarenko, A. A. and A. S. Lapik (1968). Gigiena, 68:25-28.

Type:	**Dermal Sensitization (Modified Maguire Method)**
Species/Strain:	Guinea pig/Duncan Hartley
Method:	A topical application of a 0.1 mL aliquot of 4,4'-oxydianiline was applied to the clipped and depilated backs of 10 male guinea pigs (approximately 300 g) 4 times in 10 days. At the time of the 3rd application, 0.2 mL of Freund's adjuvant was injected intradermally at one point adjacent to the insult site. After a 2-week rest period, the guinea pigs were challenged on the clipped flanks with the test material on 1 flank of each animal. The challenge site was evaluated for erythema and edema at 24 and 48 hours. A moderate erythema and/or edema in 2 or more guinea pigs was considered sufficient to classify the test material as a potential human skin sensitizer. Ten additional guinea pigs were treated with the diglycidyl ether of 2,2-di-(p,p'-hydroxyphenyl)propane, which served as a positive control.
GLP:	Unknown
Test Substance:	4,4'-Oxydianiline, purity not specified

Results:	4,4'-Oxydianiline caused sensitization in 6/10 guinea pigs. The positive control material produced sensitization, thus validating the method.
Reference:	Rao, K. S. et al. (1981). Drug Chem. Toxicol., 4(4):331-351.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Dermal Sensitization: None Found.

Type:	**Eye Irritation**
Species/Strain:	Male rabbits/Albino
Method:	A dose of 10 mg of solid 4,4'-oxydianiline was placed into the right conjunctival sac of each of 2 rabbits (age not specified). After 20 seconds, 1 treated eye was washed with tap water for 1 minute. The treated eye of the other rabbit was not washed. Observations of the cornea, iris, and conjunctiva were made with an ophthalmoscope at 1 and 4 hours, and at 1, 2, and 3 days. Eyes were stained and a slit-lamp biomicroscope was used at examinations after the day of treatment.
GLP:	No
Test Substance:	4,4'-Oxydianiline, purity > 95%
Results:	After treatment with 4,4'-oxydianiline, washed and unwashed rabbit eyes had slight corneal clouding. The unwashed eye also displayed mild conjunctivitis. Both eyes were normal 1 day after treatment.
Reference:	DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 723-81.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Eye Irritation:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Lapik, A. S. and M. P. Dolgykh (1984). Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Biol. Nauk, (3):124-126 (CA102:199083g).

Makarenko, A. A. and A. S. Lapik (1968). Gigiena, 68:25-28.

5.2 Repeated Dose Toxicity

Type:	**2-Year Feeding Study**
Species/Strain:	Rats/F344 Mice/B6C3F1
Sex/Number:	Male and female/50 per sex per dose level
Exposure Period:	103 weeks
Frequency of Treatment:	*Ad libitum*
Exposure Levels:	Rats: 0, 200, 400, 500 ppm Mice: 0, 150, 300, 800 ppm
Method:	Rats and mice (5 and 6 weeks old at study start, respectively) were fed diets containing appropriate levels of the test substance *ad libitum* 7 days/week for 103 weeks. Rats were housed 4/cage, and mice were housed 5/cage during the study. All animals were observed twice daily for signs of toxicity. Mean body weights of animals by cage were recorded every 2 weeks for the first 13 weeks, and monthly thereafter. Clinical signs were recorded monthly. Moribund animals and animals that survived to the end of the study were killed and necropsied. Animals that were found dead were necropsied, unless precluded by autolysis or cannibalization. Examinations for grossly visible lesions were performed on major tissues or organs. Tissues were preserved, and 41 and 42 tissues were examined microscopically in rats and mice, respectively. Analyses of the stability of 4,4’-oxydianiline in feed were performed by assaying dimethyl formamide extracts from samples of diet mixtures containing 100,000 ppm that had been stored at -20º, 5º, 25º, or 45ºC for 2 weeks. The concentrations of the test substance in the extracts were determined by vapor-phase chromatography. Selected batches of the formulated diets (200 and 800 ppm) administered during the study were analyzed for accuracy of dose level by spectrophotometric analysis.
GLP:	Unknown
Test Substance:	4,4’-Oxydianiline, purity 98.9%
Results:	4,4’-Oxydianiline at 100,000 ppm was stable in feed for 2 weeks at 45ºC. However, test diets were stored at 4ºC for no longer than 1 week. The mean concentration of 12 feed samples containing a theoretical level of 200 ppm was 200 ± 29 ppm, and the mean concentration of 14 samples measured in duplicate and containing a theoretical level of 800 ppm was 780 ± 103 ppm.

Mortality of male rats was 25/50 (50%), 16/50 (32%), 15/50 (30%), and 20/50 (40%) at 0, 200, 400, and 500 ppm, respectively. Mortality of female rats was 10/50 (20%), 12/50 (24%), 16/50 (32%), and 37/50 (74%) at 0, 200, 400, and 500 ppm, respectively. In addition, female rats at 500 ppm died earlier than those in the other groups. Survival was significantly shortened in the 500 ppm female rats.

Mortality of male mice was 15/50 (30%), 11/50 (22%), 16/49 (33%), and 16/50 (32%) at 0, 150, 300, and 800 ppm, respectively. Mortality of female mice was 8/50 (18%), 17/50 (34%), 17/50 (34%), and 8/50 (16%) at 0, 150, 300, and 800 ppm, respectively. Survival was significantly shortened in the 150 and 300 ppm female mice.

A dose-related depression in mean body weight gain was observed for all groups of dosed rats and mice. Labored breathing in all female rats at 500 ppm, and a compound-related increase in the number of mice with discharging, cloudy, or swollen eyes was observed.

Hepatocellular carcinomas or neoplastic nodules occurred in male rats at incidences that were dose-related, and the incidences in all dose groups were higher than in the corresponding control groups. In female rats, hepatocellular carcinomas or neoplastic nodules occurred at incidences that were dose-related, and the incidences in the 400 and 500 ppm groups were significantly higher than those in the controls.

Dose-related incidences of follicular-cell adenomas or carcinomas of the thyroid occurred in male and female rats. The incidences in the 400 and 500 ppm groups of either sex were significantly higher than those in the corresponding control groups.

The following table contains the incidences of the above mentioned tumors in male and female rats.

Dose (ppm)	0	200	400	500
Hepatocellular carcinoma:				
Males	0/50	4/50	23/50	22/50
Females	0/50	0/49	4/50	6/50
Hepatocellular neoplastic nodule:				
Males	1/50	9/50	18/50	17/50
Females	3/50	0/49	20/50	11/50
Hepatocellular carcinoma or neoplastic nodule:				
Males	1/50	13/50	41/50	39/50
Females	3/50	0/49	24/50	17/50
Thyroid follicular cell adenoma:				
Males	1/46	1/47	8/46	13/50
Females	0/49	2/48	17/48	16/50
Thyroid follicular cell carcinoma:				
Males	0/46	5/47	9/46	15/50
Females	0/49	2/48	12/48	7/50
Thyroid follicular cell adenoma or carcinoma:				
Males	1/46	6/47	17/46	28/50
Females	0/49	4/48	29/48	23/50

Except for focal mineralization of the kidney and transitional cell hyperplasia of the renal pelvis in a few treated rats, there were no other test substance-related non-neoplastic lesions. The following table contains incidences of the above mentioned non-neoplastic lesions:

Kidney mineralization:				
Males	0/50	1/50	0/50	11/50
Females	3/49	10/50	7/50	16/49
Epithelial hyperplasia of renal pelvis:				
Males	1/50	2/50	4/50	7/50
Females	0/49	2/50	5/50	4/49

Significantly increasing trends in harderian gland adenomas were observed in male mice, and the incidences in all dosed groups were significantly higher than the incidence in the control group. This same type of neoplasm occurred in females of all doses at incidences that were significantly higher than those in the controls.

Hepatocellular adenomas or carcinomas in the 150 ppm male mice occurred with an incidence that was significantly higher than that found in the control. In female mice, these kinds of tumors occurred with a dose-related trend that was significant, and the incidence in the 800 ppm group was significantly higher than that of the controls.

Follicular-cell adenomas in the thyroid occurred with a positive trend in female mice, and the incidence in the 800 ppm group was also significantly higher than that in the controls.

Adenomas in the pituitary occurred in male mice with a positive trend, and the incidence in the 800 ppm group was higher than in the controls; however, the p-values were above the level of significance required when the Bonferroni inequality criterion was used.

Hemangiomas of the circulatory system occurred in male mice with a dose-related trend that was significant. Incidences in the 300 and 800 ppm groups were significantly higher than in the controls; however, the p-values were above the level of significance required when the Bonferroni inequality criterion was used.

The following table contains the incidences of the above mentioned tumors in male and female mice.

Dose (ppm)	0	150	300	800
Harderian Gland adenomas:				
Males	1/50	17/50	13/49	17/50
Females	2/50	15/50	14/50	12/50
Hepatocellular adenomas:				
Males	11/50	13/50	11/49	10/50
Females	4/50	6/49	9/48	14/50
Hepatocellular carcinomas:				
Males	18/50	27/50	23/49	26/50
Females	4/50	7/49	6/48	15/50
Hepatocellular adenomas or carcinomas:				
Males	29/50	40/50	34/49	36/50
Females	8/50	13/49	15/48	29/50
Thyroid follicular cell adenomas:				
Females	0/46	0/43	0/42	7/48
Pituitary adenomas:				
Males	1/37	0/44	0/34	7/35
Circulatory System hemangiomas:				
Males	0/50	0/50	5/49	5/50

Other non-neoplastic lesions occured both in control and treated mice, but none of them appeared to be treatment related.

Under the conditions of this study, the test substance was carcinogenic to F344 rats and B6C3F1 mice, causing both increased incidences of follicular-cell neoplasms of the thyroid gland and liver neoplasms in rats, as well as an increased incidence of neoplasms of the liver, harderian gland, and thyroid gland in mice.

References:	NCI (National Cancer Institute) (1980). Technical Report Series No. 205, National Institutes of Health, Bethesda, MD. Murthy, A. S. K. and G. Snow (1980). Proc. Am. Assoc. Cancer Res., 21:118 (Abstract No. 474). Murthy, A. S. K. et al. (1985). J. Natl. Cancer Inst., 74(1):203-208. Weisburger, E. K. et al. (1984). J. Natl. Cancer Inst., 72(6):1457-1463. Weisburger, E. K. (1983). NTIS Pub. PB83-220137.
Reliability:	High because a scientifically defensible or guideline method was used.
Type:	**2-Year Feeding Study**
Species/Strain:	Rats/ChR-CD
Sex/Number:	Male and female/60 per sex per dose level
Exposure Period:	23 Months
Frequency of Treatment:	*Ad libitum*
Exposure Levels:	0, 200, 400 ppm
Method:	Rats (age not specified) were fed diets containing appropriate levels of the test substance *ad libitum* 7 days/week for 23 months. All rats were weighed once/week during the 1st 6 months, biweekly for the next 6 months, and every 4th week for the remainder of the study. During the test, rats were observed daily for abnormal behavior and clinical manifestations of toxicity. Food consumption was determined on a group basis at each weighing period, and food efficiency and average daily intake of 4,4'-oxydianiline were calculated. Diets were prepared fresh each week for the 1st 38 weeks, and thereafter approximately every 2-3 weeks. The diets were stored under refrigeration until used. Samples of control and test diets were collected during the study and analyzed. The samples included freshly mixed diets, diets exposed to room temperature for 24 hours, and diets stored under refrigeration for 7 days, at each dose level. After 1, 2, 3, 6, 9, 12, 18, and 23 months of continuous feeding, blood was collected from 10 male and 10 female rats from each dietary level, and 7 hematologic parameters were measured or calculated. At the same time intervals,

rats used for observation of hematology parameters, were placed in metabolism cages for 48 hours. The urine collected during the 2nd 24-hour interval was analyzed for 12 urine chemistry parameters. Alkaline phosphatase (AP) activity and glutamic-pyruvic transaminase (GPT) activity were determined on blood samples taken from 10 male and 10 female rats (not those designated for hematology and urinary analysis) at the same time intervals stated above. At 1, 2, 3, 6, 9 (females only), and 23 months, gamma-glutamyl transpeptidase (gGT) activity in these samples was also measured.

After 1-year of continuous feeding, 10 rats from each group were sacrificed for gross and histopathologic evaluation. After 23 months, all surviving rats were sacrificed for similar evaluation. At the earlier sacrifice, 11 organs were weighed for the control and 400 ppm dose groups, and organ weight/body weight ratios were calculated. At 23 months, these weights and ratios were recorded for all surviving animals. Histological examination was performed on 35 tissues from the control and 400 ppm dose groups, and 4 tissues from the 200 ppm dose groups.

An ophthalmoscopic examination was conducted on selected survivors after approximately 84 and 100 weeks on test. Both eyes were examined by focal illumination, indirect ophthalmoscopy, and when necessary, slit-lamp microscopy.

GLP: No

Test Substance: 4,4’-Oxydianiline, purity 97%

Results: Diet samples were analyzed for 4,4’-oxydianiline content after approximately 18 months of continuous feeding, and at the termination of the study. At the earlier sampling, the actual 4,4’-oxydianiline content (corrected for 97% active ingredient) was 100 and 89% for the 200 and 400 ppm dietary levels, respectively. At the latter sampling, the respective values (corrected for 97% active ingredient) were 89 and 93% of the nominal dietary levels.

At the end of the study, the survival of the 400 ppm males was significantly decreased, while the survival of the 400 ppm females was significantly increased. The average final body weights of male (200 and 400 ppm) and female (400 ppm) rats were significantly decreased. Female rats fed 400 ppm 4,4’-oxydianiline consumed slightly less food than females fed control or 200 ppm diets; the latter consumed slightly less food than control females, from 6 months to

1 year on test. There was an apparent dose-related decrease in the time to 1st observance of eye-related clinical signs, which included corneal opacity, pale eye, and apparent blindness.

Small, but significant depressions of erythrocyte count, relative number of eosinophils, hemoglobin, hematocrit, and mean corpuscular hemoglobin concentration occurred in male rats fed 400 ppm 4,4'-oxydianiline. A significant depression of hemoglobin occurred in male rats fed 200 ppm. Slight decreases in mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration suggested a similar effect on the erythrocytes in treated females. Additionally, females fed 400 ppm 4,4'-oxydianiline had a significantly lower relative number of eosinophils. Male rats fed 400 ppm 4,4'-oxydianiline excreted a larger volume of more dilute urine than control males. Elevations, although not statistically significant, were observed in alkaline phosphatase, glutamic-pyruvic transaminase, and/or gamma-glutamyl transpeptidase activities of treated males and females; these elevations were more frequent at the 400 ppm level.

At the 1-year interim sacrifice, statistically significant decreased absolute thymus and heart weights, and increased relative testes and liver weights were observed in male rats fed 400 ppm 4,4'-oxydianiline. Female rats fed 400 ppm for 1 year showed a statistically significant decrease in the following absolute organ weights: lungs, thymus, heart, stomach, and liver. These females had increased relative lung, heart, liver, kidney, adrenal, and brain weights. The decreases in absolute organ weights, with the corresponding increases in relative organ weights for lungs, heart, and liver were related to a marked (26%) difference in the final body weights of females fed 400 ppm 4,4'-oxydianiline, compared to control females.

At the 1-year interim sacrifice, test substance-related changes were found in the livers of rats fed 400 ppm; the effect was more pronounced in males than in females. The focal hepatocyte alteration was considered to be degenerative and regenerative, not neoplastic. One male rat at 400 ppm had a hepatocellular carcinoma, and 1 male and 1 female rat had cholangiofibrosis. Two of 13 male rats fed 400 ppm showed retinopathy; none of 12 control males were affected. Seven of 12 females at 400 ppm had similar lesions, compared to 1 of 13 control females. Other lesions noted at the end of 1 year were considered due to natural caused and not related to the feeding of the test substance.

At the final sacrifice (23 months), there was a statistically significant decrease in the absolute lung and stomach weights of male rats fed 400 ppm 4,4'-oxydianiline. The relative lung weights of males fed 200 ppm and the relative thymus weights of males fed 400 ppm were increased. The relative weights of the following organs were significantly increased at both 200 and 400 ppm, in a dose-related fashion: heart, spleen, liver, and brain. The absolute stomach weights of females fed 200 or 400 ppm 4,4'-oxydianiline for 23 months were statistically decreased; the relative liver, kidney, and brain weights were statistically increased for females fed 400 ppm.

At the final sacrifice, significantly more liver disease (focal angiectasis and/or focal hepatocyte alteration) was observed in test animals, and the dose-response trends within each sex were significant. A slightly higher (not statistically significant) incidence of liver tumors was observed in treated rats. When time of death and time of tumor detection were analyzed, male rats fed 200 or 400 ppm 4,4'-oxydianiline had significantly higher incidence rates of testicular tumors. When the rates were not age-adjusted, the tumor incidences for individual exposure groups were not significantly different from that of the control group; however, the tumor incidence for the pooled exposure group was significantly higher than that for the control group. The dose-response trend was significant. Male rats fed 200 ppm 4,4'-oxydianiline had a higher incidence of testicular arteritis and focal interstitial cell hyperplasia than the controls; however, the incidence of these lesions in males fed 400 ppm was comparable to that in control males.

The incidence of uterine carcinoma was significantly higher in females fed 400 ppm 4,4'-oxydianiline than in controls with a significant dose-response.

Significantly more diffuse retinopathy (1 or both eyes) was observed in males and females fed 400 ppm 4,4'-oxydianiline. The dose-response trends were significant. Cataracts were also seen, usually in eyes with severe, diffuse retinopathy, and the occurrence was related to high-level 4,4'-oxydianiline exposure in both sexes.

The following table contains the incidences of the above mentioned tumors in male and female rats.

Dose (ppm)	0	200	400
Liver: Angiectasis and/or focal hepatocyte alteration:			
Males	22/49	31/57	50/57
Females	7/59	19/60	40/59
Liver: Angiectasis and/or focal hepatocyte alteration (moderate or marked degree):			
Males	9/49	20/57	44/57
Females	0/59	2/60	24/59
Liver: Hepatocellular adenoma (neoplastic nodule):			
Males	1/60	3/60	2/60
Females	1/60	0/60	1/60
Liver: Hepatocellular carcinoma:			
Males	1/60	1/60	3/60
Females	0/60	0/60	0/60
Liver: Hemangiosarcoma:			
Males	0/60	0/60	0/60
Females	1/60	2/60	0/60
Diffuse retinopathy (one eye only):			
Males	0/49	0/50	6/47
Females	0/49	0/52	3/54
Diffuse retinopathy (both eyes):			
Males	0/47	0/42	28/44
Females	0/48	0/43	40/47
Testis: Arteritis			
Males	14/55	26/58	13/56
Uterus: Adenocarcinoma:			
Females	2/60	2/60	8/60

	Uterus: Adenocarcinoma/squamous cell carcinoma:			
	Females	0/60	1/60	1/60
	Uterus: Polyp:			
	Females	0/60	3/60	3/60
	Testis: Interstitial (Leydig) cell adenoma:			
	Males	0/60	5/60	5/60
	Testis: Interstitial (Leydig) cell carcinoma:			
	Males	1/60	0/60	1/60

	Other pathological lesions noted after 23 months (final sacrifice) of continuous feeding were considered due to natural causes, and not related to the feeding of the test substance.
Reference:	DuPont Co. (1978). Unpublished Data, Haskell Laboratory Report No. 294-78. Kaplan, A. M. et al. (1980). Toxicol. Appl. Pharmacol., Suppl., A140 (Abstract No. 420).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Repeated Dose Toxicity:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

NCI (1980). National Cancer Institute, Technical Report Series No. 205, National Institutes of Health, Bethesda, MD.

Dzhioev, F. K. (1975). Vopr. Onkol., 21(3):69-73.

Griswold, D. P., Jr. et al. (1968). Cancer Res., 28(5):924-933 (CA69:9358e).

Hayden, D. W. et al. (1978). Vet. Pathol., 15:649-662.

Kondratyuk, V. A. et al. (1986). Gig. Sanit., 0(6):31-34 (CA105:92614r).

Lapik, A. S. et al. (1968). Hyg. Sanit., 33(10):137-138.

Lapik, A. S. and M. P. Dolgky (1984). Izv. Sib. Otd. Akad. Nauk SSSR Ser. Biol. Nauk., (3):124-126 (CA102:199083g).

Makarenko, A. A. and A. S. Lapik (1968). Gigiena, 68:25-28 (CA71:104943s).

Steinhoff, D. (1977). Naturwissenschaften, 64(7):394 (CA87:112718t).

Weisburger, E. (1983). Basic Life Sci., 24(Organ Species Specif. Chem. Carcinog.):23-47 (CA98:138762e).

Weisburger, E. K. (1983). EPA-600/9-83-008, PB83-220137 (CA100:116194d).

5.3 Developmental Toxicity: No Data.

5.4 Reproductive Toxicity

Species/Strain:	Rats/Fischer 344 and CD
Sex/Number:	Male and female (CD rats)/40 control and 20 per dose level Male (Fischer rats)/10 per dose level
Route of Administration:	Orally in feed
Exposure Period:	90 days of feeding plus 1-generation reproduction
Frequency of Treatment:	*Ad libitum* for 29 days; 2 hours daily for the remainder of the study
Exposure Levels:	0, 10, 100, 400 ppm
Method:	Weanling rats were administered 4,4'-oxydianiline in their feed for 90 days. For the 1st 29 days of the study, all rats received their respective diet *ad libitum*. However, due to concerns regarding test material stability, from test day 30 until the end of the study, the rats were given access to their respective diet from approximately 1600-1800 hours, daily. At the initiation of the study, during weeks 2, 3, 5, and at the end of the reproduction phase of the study, samples were collected and analyzed. During the 90-day feeding phase, body weights, clinical observations, and individual food consumption were recorded. After approximately 90 days of continuous feeding, all Fischer 344 rats were sacrificed and the reproductive tracts were examined for gross abnormalities. Testes with epididymides were weighed and relative testes weights were calculated. Tissues processed for histopathological examination consisted of testis, epididymis, prostrate

(ventral and dorsal), seminal vesicle, coagulating gland, ampullary gland, and urinary bladder.

Following the 90-day feeding study, all surviving CD rats (F_0) were used in a 1-generation, 2-litter reproduction study. The rats were mated in the following manner: 10 males from the 10, 100, and 400 ppm groups were mated to untreated females; 10 females from the 10, 100, and 400 ppm groups were mated to untreated males; 10 untreated males were mated to 10 untreated females; and 10 males from the test groups were mated to females from the corresponding test groups. During the 1st 15-day mating phase, each female was housed with 1 male. After completion of the mating phase, female rats were separated from male rats and individually housed. Six days after separation from the males, the females were examined twice daily for birth of young (F_{1A}). On day 4 postpartum, the litters were culled randomly to 10. Remaining pups were sacrificed and did not receive pathological evaluation. Weanlings (21 days after birth) were weighed, sexed, and sacrificed, and did not receive pathological evaluation. Pups that died prior to weaning or F_0 females that died during the reproduction phase of the study did not receive pathological evaluation.

Approximately 1 week after weaning the last F_{1A} litter, the F_0 females were mated again in the same manner as described above, but to different F_0 males (from the same group as previous mating) to produce the F_{1B} litters. During the 15-day mating period, each female was checked daily for the presence of copulation plugs.

Following the last mating in the reproduction phase of the study, the male F_0 CD rats were sacrificed, and testes with epididymides were weighed and the reproductive tract was examined for gross abnormalities, as described previously for the Fischer 344 rats. F_{1B} pups were treated in the same manner as the F_{1A} pups. After weaning of the F_{1B} pups, all F_0 females were sacrificed and did not receive pathological evaluation.

During the reproduction phase of the study, the following were recorded: all matings, number of females bearing litters, number of pups born and born alive, individual litter weights 24 hours and 4 days postpartum, number of pups before and after litter reduction, number of pups per litter

12 days postpartum, number and individual body weights of male and female pups at weaning, and body weights of F_0 female rats at the time of weaning of their pups. The following reproduction and lactation parameters were determined: fertility, gestation, viability, and lactation indices, mean number of pups per litter, percent of pups born alive, litter survival, mean pup weights per litter, and mean male and female weanling body weights per litter.

During the reproduction phase of the study all F_0 male and female rats and all litters were examined at least once daily for abnormal behavior or appearance and mortality.

GLP: Yes

Test Substance: 4,4'-Oxydianiline, purity 98.7%

Results: The concentration of 4,4'-oxydianiline in diet samples collected and frozen immediately after mixing ranged from 92-95% of the nominal dose levels. Lower concentrations, ranging from 76-92% 4,4'-oxydianiline, were detected in samples stored under refrigeration for 3 or 7 days. Concentrations detected in samples stored at room temperature for 16 hours, 24 hours, or 7 days were 80-91%, 78-87%, and 57-67%, respectively. These data suggest instability or binding of 4,4'-oxydianiline to rodent chow when diets were refrigerated or stored at room temperature. To minimize this effect, rats were fed daily from frozen diets and allowed limited access to the diets from test day 30 through the end of the study. Concentrations of 4,4'-oxydianiline measured in diet samples which simulated actual in-use conditions (i.e., frozen diets stored at room temperature for 16 hours) ranged from 80-91% of the nominal diet concentrations. Since diet samples were not analyzed for impurities, the available data are insufficient to determine if the differences between nominal and analytical values of 4,4'-oxydianiline were due to instability or binding of the test material to rodent chow.

No mortalities occurred during the 90-day feeding phase. During the 90-day feeding phase, growth of females in the 10 ppm group and all male treated CD and Fischer 344 rats was comparable to that of their respective control groups. Female rats in the 100 ppm group exhibited a slight decrease in body weight gain. 4,4'-oxydianiline at 400 ppm interfered with the growth of female CD rats as evidenced by decreased mean body weights, weight gain, and food efficiency values. No abnormalities in appearance or behavior were observed in CD or Fischer 344 rats in the control and test groups.

Although a statistically significant decrease in mean absolute testes weights was observed in Fischer 344 rats fed diets that contained 400 ppm 4,4'-oxydianiline, no gross or histomorphological abnormalities that could be attributed to the test substance were observed in these tissues. In view of the small magnitude of weight change and lack of correlating gross or histomorphological alterations, the biological significance of the decreased testes weights in the Fischer 344 rats was unclear.

Mean absolute and relative testes weights of male CD rats in test groups were comparable to those of the control group. No test substance-related pathological abnormalities in the testes from male CD rats were observed.

Dietary administration of 4,4'-oxydianiline to male CD rats had no adverse effect on reproductive function. In female CD rats, the dietary administration of 400 ppm 4,4'-oxydianiline adversely influenced reproduction/lactation performance as evidenced by decreased mean number of pups per litter and decreased mean female weanling body weight per litter. No significant differences were observed in fertility index, gestation index, viability index, percent pups born alive, and litter survival. No remarkable clinical observations were observed during the reproduction substudy in either the F_0 parents or pups. The maternal and offspring no observable adverse effect level (NOAEL) of 4,4'-oxydianiline was 100 ppm. The maternal and offspring lowest observable adverse effect level (LOAEL) for 4,4'-oxydianiline was 400 ppm.

Reference: DuPont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 441-82.

Reliability: High because a scientifically defensible or guideline method was used.

Additional References for Reproductive Toxicity:

Data from these additional sources were not summarized because the study design was not adequate.

DuPont Co. (1978). Unpublished Data, Haskell Laboratory Report No. 294-78.

NCI (1980). National Cancer Institute, Technical Report Series No. 205, National Institutes of Health, Bethesda, MD.

5.5 Genetic Toxicity

Type:	***In vitro* Bacterial Reverse Mutation Test**
Tester Strain:	*Salmonella typhimurium* TA97, TA98, TA100, TA1535, and TA1537
Exogenous Metabolic Activation:	With and without Aroclor®-induced rat and hamster liver S-9
Exposure Concentrations:	0, 3, 10, 33, 100, 333, 1000, 3333, 10,000 µg/plate
Method:	The preincubation assay was performed as described in Haworth, S. et al. (1983). Environ. Mutagen., 5(Suppl. 1):3-142, with some differences. The test substance, *Salmonella* culture, and S-9 mix (10% rat or hamster liver) or buffer were incubated at 37°C, without shaking, for 20 minutes. The top agar was added and the contents of the tubes were mixed and poured onto the surface of petri dishes containing Vogel-Bonner medium. The histidine-independent (his+) colonies arising on these plates were machine counted following 2 days incubation at 37°C. Plates were machine counted unless precipitate was present that interfered with the count, or the color of the test substance on the plate reduced the contrast between the colonies and the background agar. At the discretion of the investigators, plates with low numbers of colonies were counted by hand. The test substance was tested initially in a toxicity assay to determine the appropriate dose range for the mutagenicity assay. The toxicity assay was performed using TA100 or the system developed by Waleh, N. S. et al. (1982). Mutat. Res., 97:247-256. Toxic concentrations were those that produced a decrease in the number of his+ colonies, or a clearing in the density of the background lawn, or both. At least 5 doses of the test substance were tested in triplicate. Experiments were repeated at least 1 week following the initial trial. A maximum of 0.05 mL solvent was added to each plate. Concurrent solvent and positive controls were run with each trial. The positive controls in the absence of exogenous metabolic activation were sodium azide (TA1535 and TA100), 9-aminoacridine (TA97 and TA1537), and 4-nitro-o-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

	A test substance was judged mutagenic or weakly mutagenic if it produced a reproducible dose-related response over the solvent control in replicate trials. A test substance was judged questionable if the results of individual trials were not reproducible, if increases in his+ revertants did not meet criteria for a weakly mutagenic response, or if only single doses produced increases in his+ revertants in repeat trials. A test substance was judged nonmutagenic if it did not meet the criteria for a mutagenic or questionable response.
GLP:	Unknown
Test Substance:	4,4'-Oxydianiline, purity 98.9%
Results:	Positive
Remarks:	4,4'-Oxydianiline was mutagenic, with activation, in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537. Although 4,4'-oxdianiline was considered to be nonmutagenic without activation, a possible indication of a very weak dose-response was observed in TA98. Precipitation in the 2 highest dose-levels limited the analysis to dose levels of ≤ 1000 μg/plate.

Mutagenic responses for individual strains are listed in the tables below.

	TA100		
Dose (μg/plate)	NA	10% HLI	10% RLI
0	142 (16.0)[b]	158 (12.7)	149 (5.9)
3	-[c]	618 (30.2)	189 (17.9)
10	-	1285 (52.4)	327 (6.7)
33	-	2149 (26.6)	579 (12.7)
100	157 (11.3)	2553 (14.5)	1059 (22.8)
333	171 (14.7)	2714 (92.0)	1566 (53.9)
1000	215 (5.5)	-	-
3333	214p (3.9)	-	-
10,000	220p (6.5)	-	-

[a] Standard error of the mean (SEM) is reported in paranthesis.
[c] No data

	TA1535		
Dose (µg/plate)	NA	10% HLI	10% RLI
0	25 (3.5)	21 (0.0)	17 (1.3)
3	-	-	-
10	-	18 (2.8)	-
33	-	31 (0.7)	-
100	26 (2.4)	55 (9.3)	17 (0.9)
333	23 (2.0)	125 (7.2)	17 (1.2)
1000	26 (2.1)	153 (15.8)	23 (1.5)
3333	16p (0.7)	-	20p (3.6)
10,000	16p (2.7)	-	17p (3.8)

	TA1537		
Dose (µg/plate)	NA	10% HLI	10% RLI
0	-	12 (2.2)	14 (3.9)
3	-	13 (2.6)	-
10	-	14 (2.9)	13 (2.0)
33	-	17 (1.5)	13 (2.1)
100	-	23 (2.5)	13 (1.0)
333	-	29 (3.3)	15 (1.2)
1000	-	-	11 (2.1)
3333	-	-	-
10,000	-	-	-

	TA97		
Dose (µg/plate)	NA	10% HLI	10% RLI
0	148 (6.9)	185 (1.8)	189 (5.5)
3	-	334 (13.0)	-
10	-	625 (43.2)	224 (20.1)
33	-	1163 (43.8)	268 (11.1)
100	172 (5.9)	1582 (16.7)	436 (2.6)
333	164 (12.1)	1718 (7.4)	584 (19.9)
1000	163 (6.2)	-	699 (52.1)
3333	130p (3.8)	-	-
10,000	145p (6.0)	-	-

	TA98		
Dose (µg/plate)	NA	10% HLI	10% RLI
0	24 (2.0)	44 (3.2)	31 (1.2)
3	-	80 (3.5)	-
10	-	177 (7.4)	46 (5.4)
33	-	507 (47.0)	71 (3.5)
100	30 (5.5)	1097 (70.8)	133 (20.3)
333	25 (3.8)	1564 (61.0)	226 (35.6)
1000	34 (1.8)	-	305 (24.5)
3333	24p (3.6)	-	-
10,000	25p (3.3)	-	-

Reference:	Zeiger, E. et al. (1988). Environ. Mol. Mutagen., 11(Suppl. 12):1-158.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for *In vitro* Bacterial Reverse Mutation Assay:

Data from the following sources support the study results above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

DuPont Co. (1979). Unpublished Data, Haskell Laboratory Report No. 341-79.

Endo, O. et al. (1984). Mutat. Res., 130:361 (Abstract No. 2).

Hayashi, K. (1987). Jpn. J. Ind. Health, 29:480-485.

Ino, T. et al. (1984). Mutat. Res., 130:365 (Abstract No. 13).

Lavoie, E. et al. (1979). Mutat. Res., 67:123-131.

Parodi, S. et al. (1981). Carcinogenesis, 2(12):1317-1326.

Shimizu, H. et al. (1982). Sangyo Igaku, 24(5):498-503 (CA98:66866s).

Shimizu, H. et al. (1976). Sangyo Igaku, 18(2):138-139 (CA98:66866s).

Takahashi, A. and H. Ono (1993). Chemistry Express, 8(9):785-788.

Takemura, N. and H. Shimizu (1978). Mutat. Res., 54:256-257.

Tanaka, K. et al. (1985). Mutat. Res., 143:11-15.

Data from this additional source were not summarized because the study design was not adequate.

Gee, P. et al. (1998). Mutat. Res., 412:115-130.

Type:	***In vitro* Chromosome Aberration and Sister Chromatid Exchange Tests**
Cell Type:	Chinese Hamster Ovary (CHO) cells
Exogenous Metabolic Activation:	With and without Aroclor®-induced rat liver homogenate S-9

Exposure Concentrations:	Chromosome Aberration without activation: 0, 50, 100, 160, 500, 1000, 1600, 2000, 3000 µg/mL Chromosome Aberration with activation: 0, 160, 500, 1000, 1600, 2000, 3000, 4000, 5000 µg/mL Sister Chromatid Exchange without activation: 0, 5, 16, 50 µg/mL Sister Chromatid Exchange with activation: 0, 160, 500, 1600, 2000, 3000, 4000, 5000 µg/mL
Method:	Chinese Hamster Ovary (CHO) cells, up to 15 passages since cloning, were used for the testing. The test substance was handled under yellow light, and dissolved in dimethyl sulfoxide (DMSO). Stock solutions were prepared at 500 mg/mL or at a lower concentration that gave a clear solution. Serial dilutions were prepared to achieve desired final concentrations by additions of 0.01 mL of the stock solution in the culture flasks. Concurrent solvent and positive controls were conducted with each test. Ten or 11 dose levels, at half-log intervals beginning at a high dose of 5 mg/mL (or as limited by solubility) were used for the 1st trial of the sister chromatid exchange (SCE) study. The dose levels for the chromosome aberration (ABS) study were chosen based on the toxicity of the test substance observed in the SCE study. *Protocol for SCE study:* Approximately 24 hours prior to cell treatment, $1x10^6$ cells were seeded per 75 cm^2 flask. A culture was established for each dose both with and without exogenous metabolic activation. For assays without metabolic activation, the medium was replaced with fresh medium immediately before treatment with the test substance. Cells were treated with test or control substances for 2 hours to allow interaction with cells before the addition of bromodeoxyuridine (BrdUrd). BrdUrd was then added, and incubation was continued for an additional 24 hours. The medium was removed, and fresh medium containing BrdUrd and colcemid was added and incubation was continued for 2-3 hours. For assays with exogenous metabolic activation, the cells were rinsed twice, after which culture medium without fetal bovine serum (FBS) was added. Cells were

incubated for 2 hours in the presence of the test or control substance and the S-9 reaction mixture. After the 2 hour exposure period, cells were washed twice, and then complete medium was added. Cells were incubated for an additional 26 hours, with colcemid present for the final 2-3 hours of incubation.

Two to 3 hours after addition of colcemid, cells were harvested by mitotic shake-off. Prior to harvesting, the percent confluency in each flask was estimated. Harvested cells were treated for about 3 minutes at room temperature with hypotonic KCl, washed with fixative, dropped onto slides, air dried, and stained by a modified fluorescence pulse Giemsa (FPG) technique, described in Goto, K. et al. (1978). Chromosoma, 66:351-359. Fifty 2nd-division metaphase cells were scored per dose for the incidence of SCE. The number of chromosomes in each cell was also recorded. Any cell that had fewer than 19 or more than 23 chromosomes was excluded.

Protocol for ABS study:

Approximately 24 hours prior to cell treatment, 1.2×10^6 cells were seeded per 75 cm^2 flask. A culture was established for each dose both with and without exogenous metabolic activation. For assays without metabolic activation, the testing approach was similar to the corresponding SCE study, except that cells were treated for about 10 hours and BrdUrd was omitted. Colcemid was added 2-3 hours prior to cell harvest by mitotic shake-off.

The test protocol for assays with exogenous metabolic activation was also similar to the corresponding SCE studies except that BrdUrd was omitted and cells were harvested approximately 11 hours after removal of the S-9 fraction. Colcemid was added 2 hours prior to harvest. Slides were stained with Giemsa, and 100 cells were scored for each dose. Only metaphase cells in which the chromosome number was between 19 and 23 were scored. The chromosome number was recorded for each cell and chromosome or chromatid type aberrations were classified into 3 categories: simple (breaks, fragments, double minutes), complex (interchanges, rearrangements), and other (pulverized, more than 10 aberrations/cell).

Positive results in initial tests were confirmed by additional tests. If both –S-9 and +S-9 studies gave a positive response and required confirmation, they were done sequentially (-S-9 first). If the –S-9 repeat was positive, the repeat +S-9 study was not always performed.

The standard time for obtaining 2nd-division metaphase cells in SCE studies was 26 hours after adding BrdUrd. If the test substance caused cell cycle delay, harvest times were extended, generally in 5-hour increments, with colcemid present for the last 2 hours. For ABS tests, harvest times were similarly extended based on the observation of cell cycle delay in the SCE trials.

GLP: Unknown

Test Substance: 4,4'-Oxydianiline, purity 98.9%

Results: Positive

Remarks: 4,4'-Oxydianiline caused a significant increase in the incidence of SCE as well as ABS in CHO cells, both in the presence and absence of exogenous metabolic activation. The percent cells with aberrations and the number of SCEs are listed in the tables below:

		Percent Cells with Aberrations		
Dose (µg/mL; -S9)	Cells	Total	Simple	Complex
Harvest Time: 12 hrs				
0	100	1.00	1.00	0.00
50	100	2.00	2.00	0.00
160	100	9.00	2.00	6.00
500	100	3.00	2.00	1.00
1000	100	1.00	0.00	0.00
1600	100	6.00	4.00	3.00
2000	100	1.00	1.00	0.00

		Percent Cells with Aberrations		
Dose (µg/mL; -S9)	Cells	Total	Simple	Complex
Harvest Time: 14 hrs				
0	100	4.00	2.00	2.00
100	100	13.00	13.00	2.00
500	100	16.00	16.00	2.00
1000	100	24.00	22.00	6.00
2000	100	11.00	8.00	2.00
3000	100	17.00	8.00	10.00

Dose (µg/mL; -S9) Harvest Time: 17.5 hrs	Cells	Percent Cells with Aberrations Total	Simple	Complex
0	100	4.00	2.00	2.00
100	100	12.00	7.00	5.00
500	100	32.00	18.00	16.00
1000	66	42.00	23.00	17.00
2000	100	24.00	11.00	17.00
3000	100	16.00	10.00	8.00

Dose (µg/mL; +S9) Harvest Time: 12 hrs	Cells	Percent Cells with Aberrations Total	Simple	Complex
0	100	3.00	2.00	2.00
160	100	4.00	2.00	2.00
500	100	16.00	9.00	6.00
1600	100	18.00	10.00	13.00
5000	100	9.00	5.00	4.00

Dose (µg/mL; +S9) Harvest Time: 13 hrs	Cells	Percent Cells with Aberrations Total	Simple	Complex
0	100	1.00	0.00	1.00
500	100	7.00	4.00	3.00
1000	100	7.00	5.00	3.00
2000	100	13.00	7.00	8.00
3000	100	15.00	6.00	8.00
4000	100	16.00	9.00	7.00
5000	100	20.00	5.00	15.00

Dose (µg/mL; +S9) Harvest Time: 16.5 hrs	Cells	Percent Cells with Aberrations Total	Simple	Complex
0	100	1.00	0.00	1.00
500	100	4.00	3.00	2.00
2000	100	17.00	10.00	10.00
3000	100	10.00	8.00	4.00
4000	100	11.00	5.00	8.00
5000	100	20.00	4.00	18.00

Dose (µg/mL; -S9)	Total Chromosomes	Total SCE	SCE per Cell
0	1041	411	8.22
5	1040	426	8.52
16	1031	463	9.26
50	1039	632	12.64

Dose (µg/mL; -S9)	Total Chromosomes	Total SCE	SCE per Cell	Harvest Time
0	1030	371	7.42	26.50
50	1032	731	14.62	34.00
150	1042	1323	26.46	34.00
200	1034	1572	31.44	44.00
300	1041	1668	33.36	44.00
400	1041	1861	37.22	44.00
500	1041	1757	35.14	44.00

Dose (µg/mL; +S9)	Total Chromosomes	Total SCE	SCE per Cell
0	1040	438	8.76
160	1039	440	8.80
500	1021	475	9.50
1600	1045	582	11.64

Dose (µg/mL; +S9)	Total Chromosomes	Total SCE	SCE per Cell
0	1051	380	7.60
500	1035	463	9.26
2000	1034	448	8.96
3000	1042	552	11.04
4000	1029	551	11.02
5000	560	479	17.74

Reference: Gulati, D. K. et al. (1989). Environ. Mol. Mutagen., 13:133-193.

Reliability: High because a scientifically defensible or guideline method was used.

Type:	***In vitro* Mouse Lymphoma Forward Mutation Assay**
Cell Type:	Mouse lymphoma cells (L5178Y; TK locus)
Exogenous Metabolic Activation:	With and without Aroclor®-induced rat liver S-9 (RLI) and/or uninduced rat liver S-9 (RLN)
Exposure Concentrations:	0, 15.625, 31.25, 50, 62.5, 100, 125, 150, 200, 250, 500 µg/mL
Method:	The positive control substances used were 3-methylcholanthrene (3-MC) and ethyl methanesulphonate (EMS) for tests with and without exogenous metabolic activation, respectively. The vehicle control was the solvent for the test substance.

Each experiment, other than the initial toxicity test, normally consisted of the following groups: vehicle control, 4 cultures; positive control, 2 cultures; and at least 5 test substance concentrations, 2 cultures/concentration. The initial experiment was a toxicity test in which cell population expansion was measured. Ten-fold differences in test substance concentrations were used in the toxicity test, the highest being 5 mg/mL unless a much lower concentration was indicated by the poor solubility of a test substance. This test was followed by at least 2 experiments in the absence of S-9 mix. Test substance concentrations were primarily 2-fold dilutions from the highest testable concentration, as estimated from the toxicity test. If a clear positive response was observed in these experiments, no further testing was performed either in the absence or presence of S-9.

Each exposed culture consisted of $6x10^6$ cells in a final volume of 10 mL in a screw-cap plastic tube. This tube was incubated for 4 hours on a rotating horizontal axis roller drum. At the end of the incubation, the cells were sedimented by centrifugation, washed, and resuspended in 20 mL. These cell suspensions ($3x10^5$ cells/mL) were incubated for a 2-day expression period, the cell population density being adjusted back to 20 mL of $3x10^5$ cells/mL after 24 hours. After 48 hours, the cell population densities were estimated and culture volumes containing $3x10^6$ cells adjusted to 15 mL, giving a cell population density of $2x10^5$ cells/mL.

A 0.1 mL sample of the cell suspension was withdrawn and diluted. Three 0.1 mL samples (200 cells) of the diluted

	cultures were transferred to tubes, mixed with cloning medium containing agar, and poured onto Petri plates. Three aliquots (each containing 10^6 cells) of the remaining culture were distributed to tubes, mixed with cloning medium containing agar and trifluorothymidine, then poured onto Petri plates. The agar was gelled at 4°C for 5-10 minutes, then the plates were incubated for 11-14 days at 37°C. Colonies were counted using an automated colony counter. Toxicity was expressed as either a reduction of cell population growth in suspension during the expression period or a reduction in cloning efficiency. A measure of overall toxicity was relative total growth (RTG). A test was considered positive when, out of 3 trials, a positive trial was reproducible. A test was considered negative when, out of 3 trials, a positive response or a positive dose was not reproducible. A test was considered questionable when, out of 3 trials, neither a positive nor a negative response was produced.
GLP:	Unknown
Test Substance:	4,4'-Oxydianiline, purity not specified
Results:	Positive
Remarks:	The lowest tested concentration of 4,4'-oxydianiline, 50 µg/mL, induced statistically significant increases in the mutant fraction in the absence of S-9 mix. A dose-related response was observed over 3 concentrations before toxicity became excessive. The relative total growth (RTG) at the lowest observed effective dose (LOED) was about 88%. Because a clear positive response was observed in the absence of S-9 mix, no further testing was performed either in the absence or presence of S-9.
Reference:	McGregor, D. B. et al. (1988) Environ. Mol. Mutagen., 12(1):85-154.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for *In vitro* Genetic Toxicity Studies:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Rudd, C. J. et al. (1983). Environ. Mutagen, 5:419 (Abstract Cd-19).

Chromosome Aberration and Sister Chromatid Exchange (CHO Cells)

Lapik, A. S. et al. (1970). Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Biol. Nauk,(2):145-146 (CA74:85985a).

Syrian Hamster Embryo Cell Transformation

Tu, A. S. and A. Sivak (1985). Carcinog. Compr. Surv., Vol. 9, pp. 411-421.

Tu, S. et al. (1986). Environ. Mutagen., 8:77-98.

Hatch, G. G. et al. (1985). Environ. Mutagen., 7(Suppl. 3):75-76.

Hatch, G. G. et al. (1985). Carcinogenesis: A Comprehensive Survey, 9:437-447 (BIOSIS/86/10167).

Hatch, G. G. et al. (1986). Environ. Mutagen., 8:515-531.

UDS/ DNA Strand Breaks

Brambilla, G. et al. (1985). Carcinogenesis, 6(9):1285-1288.

Mirsalis, J. et al. (1983). Environ Mutagen., 5:482.

Mirsalis, J. C. et al. (1989). Environ. Mol. Mutagen., 14:155-164.

Mori, H. et al. (1988). Mutat. Res., 204(4):683-688.

Shaddock, J. G. et al. (1988). Environ. Mol. Mutagen, 11(Suppl. 11):93 (Abstract No. 227).

Shaddock, J. G. (1989). Environ. Mol. Mutagen., 13:281-288.

Transformation Of Balb/c-3T3 Cells

Matthews, E. J. et al. (1993). Environ. Health Persp., 101 (Suppl. 2):347-482.

Type:	***In vivo* Mouse Micronucleus Test**
Species/Strain:	Mice/B6C3F1
Sex/Number:	Male/5 per dose level
Route of Administration:	Intraperitoneal (i.p.) injection
Concentrations:	Initial Test: 0, 37.5, 75, 150 mg/kg Repeat Test: 0, 75, 150 mg/kg
Method:	Groups of 5 mice (aged 9-14 weeks, weighing 25-33 g) were administered 4,4'-oxydianiline (mixed in corn oil and

suspended with a Tek-Mar Tissumizer®) via i.p. injection at a volume of 0.4 mL per mouse on 3 consecutive days. Animals were monitored twice daily, and 48 hours after the 3rd treatment, the surviving mice were euthanized. Bone marrow smears were prepared by a direct technique, fixed, and stained with acridine orange. Bone marrow smears from each animal were evaluated at 1000x magnification using epi-illuminated fluorescence microscopy for determination of the percentage of polychromatic erythrocytes (PCE) among 200 erythrocytes. Based on the results obtained, the maximum administered dose was estimated or additional dose determination experiments were conducted to more accurately estimate the maximum dose to be tested in the primary micronucleus (MN) test. The selection of the maximum dose to be tested for MN induction was based on mortality.

For the initial MN test, groups of 5 mice were injected i.p. on 3 consecutive days with either the test substance (at 32.5, 75, or 150 mg/kg), the positive control chemical (12.5 mg/kg 7,12-dimethylbenzanthracene in corn oil), or the solvent (corn oil). Mice were euthanized 24 hours after the 3rd treatment. Bone marrow smears were prepared, fixed, and stained with acridine orange. For each animal, slides were evaluated at 1000x magnification for the number of MNPCE among 2000 PCE and for the percentage of PCE among 200 erythrocytes.

A repeat test was performed since the results from the initial test suggested a possible positive effect.

GLP:	Unknown
Test Substance:	4,4'-Oxydianiline, purity not specified
Results:	Positive
Remarks:	All animals survived, except 1 animal at the top dose of 150 mg/kg during the retest. No clinical signs of toxicity were reported. The initial test was negative by statistical trend analysis, but the MNPCE frequencies in the 37.5 and 75 mg/kg dose groups were markedly elevated. The repeat test was positive by trend analysis with the MNPCE frequency in the high dose group (150 mg/kg) elevated significantly above the control. Overall, these results were considered positive. Statistical trend reanalysis of the initial test data, omitting the high dose group, provided support to the conclusion that 4,4'-oxydianiline induces MN.

The micronucleus data analysis are listed in the table below.

Dose (mg/kg)	MN-PCE/1000 (No. animals)	Survival	% PCE
0	1.70±0.26 (5)	5/5	58.2
37.5	3.30±0.46 (5)	5/5	46.3
75	4.20±0.89 (5)	5/5	56.0
150	2.90±0.40 (5)	5/5	50.1
0	1.20±0.41 (5)	5/5	50.8
75	1.70±0.34 (5)	5/5	62.8
150	2.63±0.24 (4)	4/5	61.4

Reference: Shelby, M. D. et al. (1993). Environ. Mol. Mutagen., 21:160-179.

Reliability: High because a scientifically defensible or guideline method was used.

Type: ***In vivo* Unscheduled DNA Synthesis**

Species/Strain: Rats/Fischer-344

Sex/Number: Male/3 per dose level

Route of Administration: Oral

Concentrations: 40, 180, 725 mg/kg

Method: Male rats (180-300 g) were administered 4,4'-oxydianiline via intragastric intubation as a single bolus dissolved in corn oil. Primary hepatocyte cultures were prepared from rats as described in Mitchell, A. D. and J. C. Mirsalis (1984). Single Cell Mutation Monitoring System: Methodologies and Applications, Ansari, A. A. and F. de Serres (eds.), pp. 165-216, Plenum Pub. Corp., New York, and Mirsalis, J. C. et al. (1985). Carcinogenesis, 6:1521-1524. Livers were perfused *in situ* with a solution of ethyleneglycolbis (β-amino ethyl ether)N,N'-tetraacetic acid (EGTA) in Hanks' balanced salt solution without Ca^{+2} or Mg^{2+}, followed by a 37ºC solution of Type I collagenase in Williams' Medium E.

A single-cell suspension of hepatocytes was obtained by combing out cells from the perfused liver into a petri dish containing 37ºC collagenase solution. Cells were collected by centrifugation, resuspended in cold medium, and filtered through sterile gauze. Viability was determined using Trypan blue exclusion. In general, hepatocyte viability was not adversely affected by test substance treatment, i.e.

viability generally exceeded 70%, and attachment to the coverslips in the culture plate wells did not vary.

Approximately $6x10^5$ cells were seeded into each well of a 6-well culture plate. Each well contained a coverslip in Williams' medium E (WE) supplemented with l-glutamine, gentamycin sulfate, and fetal bovine serum. After 1.5-2.0 hours incubation in a humidified atmosphere at 37ºC, 5% CO_2, the cultures were washed to remove nonviable cells (those not attached to the coverslips).

Cultures were incubated in WE containing ^{3}H-(methyl)-thymidine for 4 hours at 37ºC and 5% CO_2, followed by 14-18 hours in WE containing unlabeled thymidine. The cultures were then washed twice with WE, followed by hypotonic treatment with sodium citrate to swell the cells, fixed in glacial acetic acid:ethanol, and washed 3-6 times with deionized water. The dried coverslips were mounted to glass slides. The slides were dipped in nuclear track emulsion diluted with deionized water, and exposed at -20ºC for 7-14 days and then developed and stained as described in Mitchell and Mirsalis, 1984.

Quantitative autoradiographic grain counting was accomplished as described by Mitchell and Mirsalis, 1984. An area of a slide was randomly selected, and 50 morphologically unaltered cells were counted using a colony counter interfaced to a computer. The highest of 2 nuclear-sized areas over the cytoplasm and adjacent to the nucleus was subtracted from the nuclear count to determine the net grains/nucleus (NG). The percentage of cells undergoing repair (%IR) was determined as the percent of those cells exhibiting 5 or more NG. Three slides were scored for each animal or concentration for a total of 150 cells per animal.

The test substance was considered negative if the NG of all dose groups was a negative number and the %IR was less than 10%. The test substance was considered positive f the average NG of any dose group exceeded 0 NG. Test substances with negative NG values, but %IR values greater than 10% were considered equivocal.

GLP:	Unknown
Test Substance:	4,4'-Oxydianiline, purity not specified
Results:	Negative

Remarks:	4,4'-Oxydianiline failed to induce unscheduled DNA synthesis in rat hepatocytes following *in vivo* treatment.
Reference:	Mirsalis, J. C. et al. (1989). Environ. Mol. Mutagen., 14:155-164.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for *In vivo* Studies:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Micronucleus

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UDS

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